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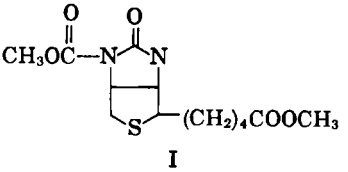
Chemical Reactivity of Models Related to a Proposed CO_2~Biotin Enzyme Complex II

By HOWARD J. SCHAEFFER and PARMATMA S. BHARGAVA

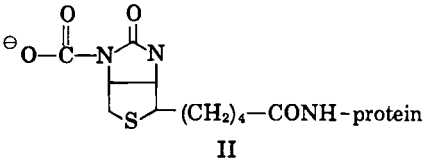
In order to gain more information about the mechanism by which biotin may function in biochemical carboxylations, a study of model compounds which are related to a proposed CO_2~biotin-enzyme complex was undertaken. The model compounds which were employed were the N-arylcarbamyl-2-imidazolidones and the N-alkyloxycarbonyl-2-imidazolidones. It has been shown that the carbonyl group of the model compounds is transferred to an attacking nucleophilic reagent. The significance of these reactions is discussed.

BIOTIN HAS BEEN the subject of numerous studies in which attempts were made to determine the biochemical functions of this vitamin; it has been observed that one of the actions of biotin is in biochemical carboxylation reactions. In the biosynthesis of fatty acids, it has been shown that a biotin-containing enzyme is involved in the carboxylation of acetyl CoA to malonyl CoA (1). Studies of fatty acid synthesis in certain cell-free extracts have demonstrated that carbon dioxide is activated by a biotin-containing enzyme and that the activated CO_2~biotin enzyme complex acts as the carboxylating reagent (2). In addition, biotin is necessary for the biosynthesis of purines since it has been observed (3) that biotin is required in the carboxylation of 5-aminoimidazole ribotide to 5-aminoimidazole-4-carboxylic acid ribotide, which after several further reactions is converted into inosinic acid.

A theory on the mechanism of biotin action has been proposed by Lynen and his co-workers from work on β-methylcrotonyl CoA carboxylase (2). These investigators found that this enzyme could not only carboxylate β-methylcrotonyl CoA but in addition could utilize free D-biotin as a substrate to give an unstable carboxybiotin. The unstable product was not isolated but after treatment with diazomethane gave the methyl ester of N-carbomethoxybiotin (I) (4). This result has led to the suggestion that the chemical structure of the CO_2~biotin enzyme complex may be represented by II.



I



II

Recently, Wakil and Waite have presented evidence that for acetyl CoA carboxylase the uriedo carbonyl group of the enzyme-bound biotin is the active carbon and that it is involved in the carboxylation reactions (5, 6). For example, it was found that in the presence of adenosine triphosphate and Mn^{2+}, acetyl CoA carboxylase incorporated C^{14}-bicarbonate to form a $C^{14}O_2$~biotin enzyme complex. Hydrolysis of this complex gave free C^{14}-biotin in which the carbon-14 was located in the ureido carbon atom (5, 6). In addition, it was also shown that growth of *Lactobacillus arabinosus* on limiting amounts of ureido-C^{14}-biotin resulted in the loss of most of the radioactivity from the biotin. Although this data is in conflict with that of Melville, Pierce, and Partridge (7), it was shown that the previous investigators had employed

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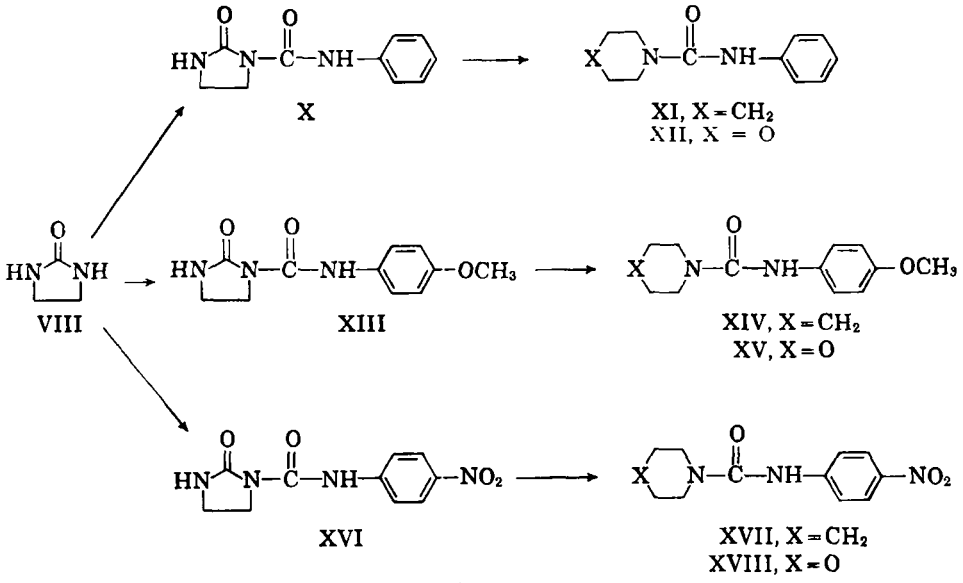
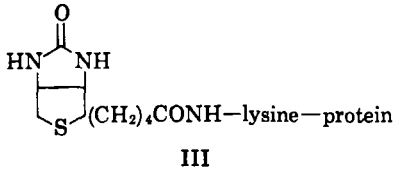
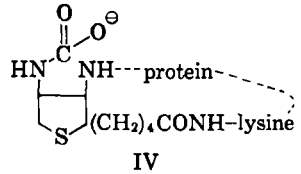


Chart I

ureido-C^{14}-biotin in an amount in excess needed for optimal growth of *L. arabinosus;* therefore, the C^{14}-biotin was stored within the cells (6). On these grounds, Wakil and Waite (5, 6) have suggested that the active form of the enzyme-biotin may be represented by either III or IV



III

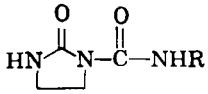


IV

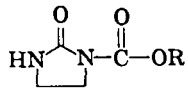
Compounds with structure  related to II or IV are known to be very unstable and undergo decarboxylation with extreme ease. It is quite possible that the actual structure of the CO_2~biotin enzyme complex is not one in which the carboxylate anion of biotin is present as such but rather one in which it has been partially stabilized by reaction with the associated protein. Thus, it appears that the carboxylation reactions may involve three steps: (*a*) formation of a CO_2~biotin enzyme complex, (*b*) partial stabilization of the complex by reaction of the carboxylate anion with a functional group of associated protein such as an amino or hydroxyl group, and (*c*) transfer of the carboxyl group to an attacking nucleophilic agent, *e.g.*, acetyl CoA which would produce the corresponding carboxylic acid and regenerate the biotin-containing enzyme.

To gain more insight into the mechanism of biotin action, a study of the transfer of a carbonyl group to an attacking nucleophilic reagent, using model compounds related to the proposed CO_2~biotin enzyme complex was undertaken. In particular, step (*c*) of the biochemical carboxylation reaction was studied.

Since it was essential to use model compounds which would not undergo spontaneous decarboxylation, certain model compounds containing an imidazolidone system were selected; the simplest model systems for such a study are the N-substituted derivatives of 2-imidazolidone (V and VI).



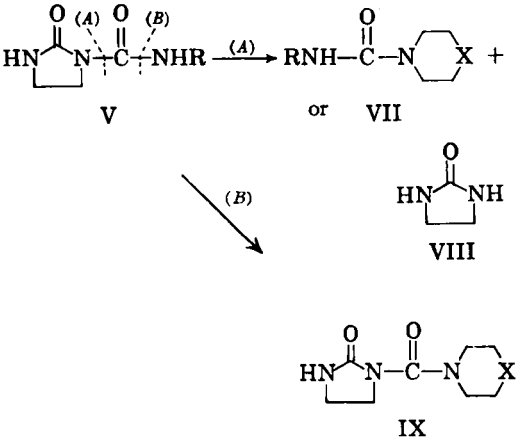
V



VI

In biochemical reactions, one of the common examples of a nucleophilic agent is acetyl CoA. Due to the presence of thiol ester group in acetyl CoA, a carbanion is generated at the α-carbon which attacks the CO_2~biotin enzyme complex to generate a carboxylic acid and the free enzyme. In our model studies morpholine and piperidine were used as the nucleophilic reagents to represent the attack of acetyl CoA on the CO_2~biotin enzyme complex.

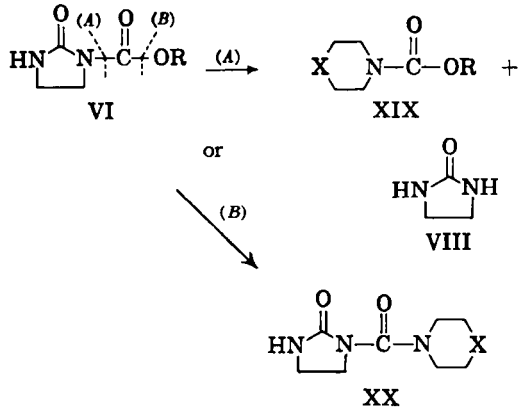
For those model compounds with structures related to V, *a priori* there are two pathways by which reaction may occur



For example, V could undergo reaction to give VII and VIII (path *A*) or by displacement to give IX (path *B*). To determine which pathway is preferred, several compounds related to V were prepared. When 2-imidazolidone was allowed to react with different isocyanates (phenyl, *p*-methoxyphenyl or *p*-nitrophenyl), moderate yields of N-phenylcarbamyl, N-*p*-methoxyphenylcarbamyl and N-*p*-nitrophenylcarbamyl-2-imidazolidone (X, XIII, XVI) were obtained (see Chart I). These model compounds had characteristic absorption in the 1735 and 1685 cm.$^{-1}$ region in the infrared spectrum indicative of carbonyl functions. When X, XIII, and XVI were allowed to react with either piperidine or morpholine at reflux temperatures, the carbonyl group was transferred to the attacking nucleophilic reagent with the formation of the corresponding ureas. Authentic samples of corresponding ureas XI, XII, XIV, XV, XVII, XVIII (see Chart I) were prepared by the reaction of different isocyanates with morpholine or piperidine.

Thus, it has been established that compounds related to a proposed CO_2~biotin enzyme complex are capable of transferring their carbonyl group. In our case, however, the reactivity of the model compounds is low and therefore cannot represent the *exact* structure of the complex which occurs in the enzymatic process; nevertheless, the principle of this type of transfer has been established.

In the second phase of our study of model compounds, we examine the reaction of compounds related to VI with nucleophilic reagents. Again, one can suggest several pathways by which these model compounds might undergo reaction, *e.g.*,



To assess the effect of stabilization of the carboxyl group *via* its ester, compounds XXI, XXV, and XXVIII were prepared by allowing 2-imidazolidone VIII to react with an equimolar concentration of the corresponding alkyl or aryl chloroformates (Chart II).

It seems likely that the model compounds of this group would also result in a similar type of product XIX as obtained from V. However, these model compounds on reaction with the nucleophilic reagents, piperidine, or morpholine, gave a variety of products. The variation in product formation was

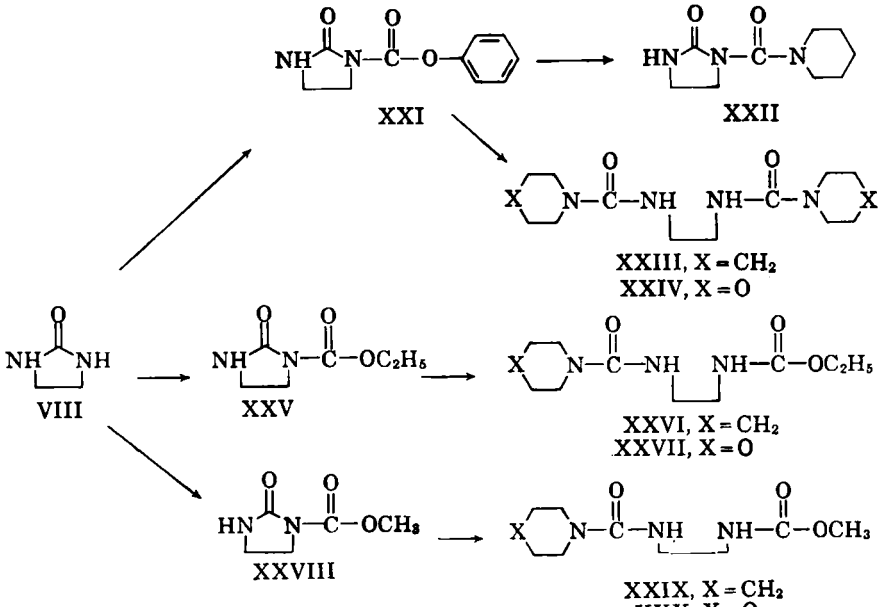


Chart II

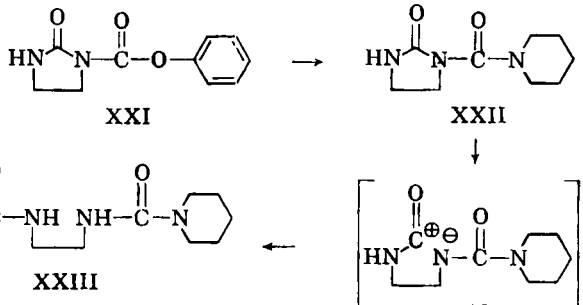
not dependent on the character of the nucleophilic reagent but rather on the ester employed.

Thus when XXI was allowed to react with piperidine or morpholine, opening of the ureido ring was observed, giving XXIII and XXIV as the major product. The reaction of compound XXI with piperidine in 1:2 molar ratio gave rise to XXII, identified as N(N-piperidinocarbonyl)-2-imidazolidone. When XXII was allowed to react with excess piperidine, XXIII was formed in good yields. However, when XXV or XXVIII was allowed to react with piperidine or morpholine, the products of reaction XXVI, XXVII, XXIX, and XXX were isolated in good yields. It was also shown that when XXVII was allowed to react with morpholine for extended times, it was converted into XXIV.

In general, the experiments were followed by a characteristic change in the infrared spectrum or by thin-layer chromatography and were found to be complete in less than 1 hour. Exceptions were noted in the case of reaction of XXI with piperidine in which it took 8 hours for complete reaction and in the case of product XXIII, 42 hours were required for its completion.

These observations suggest that the products are formed by different mechanisms. The peculiar activity of this group of model compounds indicated a second possible mechanism of action of biotin beside the N-activated carbon dioxide~biotin-enzyme complex. The opening of the ureido ring of 2-imidazolidone by nucleophilic attack suggests the possibility of transfer of ureido-carbonyl in the biological systems whenever the nucleophilic agent, *e.g.*, acetyl CoA attacks the complex.

If the following is assumed to be the mechanism of reaction of the formation of XXII and XXIII,



XXI XXII XXIII XXXI

it is possible to suggest that XXXI is the intermediate which would carry out the transfer of ureido carbonyl in different carboxylation reactions. There is a striking parallel between the result reported here from the studies of model compounds and the findings of Waite and Wakil (5, 6).

These reactions demonstrate that the transfer of a carbonyl group from model compounds, which are related to a proposed CO_2~biotin–enzyme complex, to a nucleophilic reagent can occur in two ways as shown by the model compounds V and VI. An extension of these findings is currently under investigation. The results of these studies will be the subject of a future paper.

EXPERIMENTAL[1]

N-Phenylcarbamyl-2-imidazolidone (X).—To a stirred solution of 2.58 Gm. (29.9 mmoles) of 2-imidazolidone (8) in 60 ml. of chloroform was added a 3.26-ml. (30.0 mmoles) quantity of phenyl isocyanate and 0.5 ml. of pyridine. The reaction mixture was heated under reflux for 24 hours; it was reduced to half volume *in vacuo* and allowed to crystallize. A 3.10-Gm. (50.4%) quantity of the white crystalline material, m.p. 168°, was obtained. Concentration of the mother liquor gave a second crop, 1.10 Gm. (17.9%), m.p. 168°. One recrystallization of the crude material from chloroform gave 2.80 Gm. (45.4%) of pure white crystalline product, m.p. 168–169°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3345 and 3160 (NH); 1740 and 1665 (C=O); 1625 and 1570 (NH); 1605 and 1490 (phenyl); $\lambda_{max.}^{chloroform}$ 246 mμ ($\epsilon \times 10^{-4}$) 1.40.

Anal.[2]—Calcd. for $C_{10}H_{11}N_3O_2$: C, 58.44; H, 5.35; N, 20.45. Found: C, 58.39; H, 5.30; N, 20.28.

N - p - Methoxyphenylcarbamyl - 2 - imidazolidone (XIII).—A mixture of 1.72 Gm. (19.9 mmoles) of 2-imidazolidone in 60 ml. of pyridine and 2.98 Gm. (19.9 mmoles) of *p*-methoxyphenyl isocyanate was heated under reflux for 24 hours. The reaction mixture was concentrated to half volume *in vacuo* and allowed to crystallize. A 0.840-Gm. (17.9%) quantity of the white crystalline material, m.p. 176°, was obtained. The mother liquor was evaporated *in vacuo* to dryness, and the residue on recrystallization from chloroform gave a white crystalline solid, 2.30 Gm. (49.1%), m.p. 175°. One recrystallization from chloroform gave the analytical sample, m.p. 176°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3355 and 3260 (NH); 1725 and 1685 (C=O); 1605 and 1560 (NH); 1510 (phenyl); $\lambda_{max.}^{ethanol}$ 274 mμ ($\epsilon \times 10^{-4}$) 1.20.

Anal.—Calcd. for $C_{11}H_{13}N_3O_3$: C, 56.11; H, 5.52; N, 17.86. Found: C, 56.46; H, 5.28; N, 17.86.

N - p - Nitrophenylcarbamyl - 2 - imidazolidone (XVI).—A 1.64-Gm. (9.99 mmoles) quantity of *p*-nitrophenyl isocyanate in 8 ml. of chloroform was added to a stirred solution of 0.860 Gm. (9.99 mmoles) of 2-imidazolidone in 7 ml. of chloroform. The reaction mixture was heated under reflux for 24 hours and on concentrating to half volume *in vacuo*, yellowish crystals appeared which were collected by filtration. A 1.60-Gm. (64.0%) quantity of yellowish crystalline material, m.p. 268°, was obtained. Concentration of the mother liquor gave a second crop, 0.312 Gm. (12.5%), m.p. 268–269°. The total yield was 76.5%. Two recrystallizations of the crude product from acetone gave the yellow

[1] The infrared spectra were determined on a Perkin-Elmer model 137 spectrophotometer; the ultraviolet spectra were determined on a Perkin-Elmer model 4000A spectrophotometer. The melting points were determined on a Kofler Heizbank and are corrected.

[2] The analyses reported in this paper were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

crystalline analytical sample, m.p. 270°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3230 and 3120 (NH); 1735 and 1685 (C=O); 1610 and 1565 (NH); 1595 and 1505 (phenyl); $\lambda_{max.}^{ethanol}$ 317 mμ ($\epsilon \times 10^{-3}$) 13.4.

Anal.—Calcd. for $C_{10}H_{10}N_4O_4$: C, 48.00; H, 4.02; N, 22.39. Found: C, 48.10; H, 4.12; N, 22.00.

N-p-Methoxyphenylcarbamylpiperidine (XIV).—To 1.49 Gm. (9.99 mmoles) of *p*-methoxyphenyl isocyanate was added 0.85 Gm. (9.98 mmole) of piperidine with constant stirring. The reaction was quite exothermic, and an immediate white crystalline precipitate appeared which was collected by filtration; 1.70 Gm. (72.5%), m.p. 130°. Recrystallization of the crude product from methanol and water (1:1) gave the analytical sample, m.p. 130°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3350 and 3060 (NH); 1625 (C=O); 1515 (NH); $\lambda_{max.}^{ethanol}$ 244 mμ ($\epsilon \times 10^{-3}$) 30.0.

Anal.—Calcd. for $C_{13}H_{18}N_2O_2$: C, 66.68; H, 7.68; N, 11.95. Found: C, 66.79; H, 7.74; N, 11.92.

N-Phenylcarbamylpiperidine (XI).—This compound was prepared by the procedure of Crosby and Niemann in a 66% yield, m.p. 168°, in agreement with the literature value (9).

N-p-Nitrophenylcarbamylpiperidine (XVII).—This material was prepared in a 69% yield by a modification of the procedure of Taylor, m.p. 166°, in agreement with the literature value (10).

N-p-Nitrophenylcarbamylmorpholine (XVIII).—A solution of 1.64-Gm. (10.0 mmoles) of *p*-nitrophenyl isocyanate in 10 ml. of chloroform was added to 0.871 Gm. (10.0 mmoles) of morpholine in 5 ml. of chloroform. The reaction mixture was stirred for 3 hours, then was evaporated to dryness *in vacuo*. A 2.30-Gm. (91.3%) quantity of yellow product, m.p. 216°, was obtained. Two recrystallizations of the crude product from an acetone and methanol mixture gave the analytical sample, m.p. 219°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3400 (NH); 1670 (C=O); 1590 and 1540 (phenyl); $\lambda_{max.}^{ethanol}$ 326 mμ ($\epsilon \times 10^{-3}$) 58.4.

Anal.—Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.48; H, 5.21; N, 16.68. Found: C, 52.81; H, 5.27; N, 16.67.

N-Phenylcarbamylmorpholine (XII).—This product was prepared in a 96% yield by the reaction of phenyl isocyanate with morpholine, m.p. 162°. The reported melting point for this compound is 156–159° (11).

N-p-Methoxyphenylcarbamylmorpholine (XV).—This material was prepared by the literature procedure in an 81% yield, m.p. 125°, in agreement with the literature value (12).

Reaction of N-Phenylcarbamyl-2-imidazolidone and Piperidine (Excess).—Piperidine (10.0 ml.) was added to 0.410 Gm. (1.99 mmoles) of N-phenylcarbamyl-2-imidazolidone (X). The reaction mixture was heated under reflux for 36 hours and then evaporated *in vacuo* to dryness. Recrystallization from a methanol and water mixture gave 0.200 Gm. (48.9%) of N-phenylcarbamylpiperidine (XI), m.p 168°. The identity of this product was confirmed by thin-layer chromatography (silica gel) R_f (chloroform 92.3%; acetone 7.7%) 0.80 and by mixed melting point with an authentic sample. Concentration of mother liquor gave an additional crop of crystals 0.033 Gm. (8.10%), m.p. 168°. The total yield was 57.0%.

Reaction of N-p-Methoxyphenylcarbamyl-2-imidazolidone and Piperidine (Excess).—A mixture of 0.47 Gm. (1.98 mmoles) of N-*p*-methoxyphenylcarbamyl-2-imidazolidone (XIII) and 15 ml. of piperidine was heated under reflux for 48 hours. The reaction mixture was evaporated to dryness *in vacuo*, and the residue recrystallized from a methanol and water mixture. There was obtained 0.190 Gm. (40.6%) of N-*p*-methoxyphenylcarbamyl piperidine (XIV), m.p. 130°. The identity of this product was confirmed by thin-layer chromatography (silica gel), R_f (chloroform 92.3%; acetone 7.7%) 0.65; and by mixed melting point with an authentic sample. The mother liquor on evaporating to dryness *in vacuo* and recrystallization from a methanol and water mixture gave another crop of 0.090 Gm. (19.2%), m.p. 128°. The total yield was 0.280 Gm. (59.8%).

Reaction of N-p-Nitrophenylcarbamyl-2-imidazolidone and Piperidine (Excess).—A mixture of 0.500 Gm. (2.00 mmoles) of N-*p*-nitrophenylcarbamyl-2-imidazolidone (XVI) and 15 ml. of piperidine was heated under reflux for 24 hours. The reaction mixture was evaporated to dryness *in vacuo*, and the residue after recrystallization from acetone gave 0.135 Gm. of unchanged starting material (XVI), m.p. 224°. Evaporation to dryness of the mother liquor and recrystallization of the residue from a mixture of acetone and water gave 0.248 Gm. of N-*p*-nitrophenylcarbamylpiperidine (XVII), m.p. 165°. The yield was 53.4% (based on unrecovered starting material), m.p. 165°.

Reaction of N-Phenylcarbamyl-2-imidazolidone and Morpholine (Excess).—To 0.205 Gm. (1.00 mmole) of N-phenylcarbamyl-2-imidazolidone (X) was added 7.7 ml. of morpholine. The reaction mixture was heated under reflux for 70 hours and then evaporated to dryness *in vacuo*. Recrystallization of the residue from a methanol and water mixture gave 70.0 mg. (33.9%), of N-phenylcarbamylmorpholine, m.p. 159°. The identity of this product was confirmed by thin-layer chromatography (silica gel), R_f (chloroform 92.3%; acetone 7.7%) 0.35 and by mixed melting point with an authentic sample. Concentration of mother liquor gave an additional crop of 43.0 mg. (20.8%), m.p. 159°. The total yield of XII was 113 mg. (54.8%).

Reaction of N-p-Methoxyphenylcarbamyl-2-imidazolidone and Morpholine (Excess).—To 0.117 Gm. (0.490 mmole) of N-*p*-methoxyphenylcarbamyl-2-imidazolidone (XIII) was added a 3.85-ml. quantity of morpholine. The reaction mixture was heated under reflux for 22 hours. Concentration of the reaction mixture to half volume *in vacuo* gave white crystals which were collected by filtration; 56.5 mg. (47.8%), m.p. 124°, of N-*p*-methoxyphenylcarbamylmorpholine (XV) was obtained. Evaporation of the mother liquor to dryness and recrystallization from a mixture of methanol and water gave another crop of 10.5 mg. (8.9%), m.p. 125°. The total yield of XV was 67.0 mg. (56.7%).

Reaction of N-p-Nitrophenylcarbamyl-2-imidazolidone and Morpholine (Excess).—To 0.125 Gm. (0.500 mmole) of N-*p*-nitrophenylcarbamyl-2-imidazolidone (XVI) was added 3.85 ml. of morpholine; the mixture was heated under reflux for 32 hours.

The reaction mixture was evaporated to dryness *in vacuo* and recrystallized from a methanol and acetone mixture. A 0.055-Gm. (44.0%) quantity of N-*p*-nitrophenylcarbamylmorpholine (XVIII), m.p. 216°, was obtained. The identity of the product was confirmed by thin-layer chromatography (silica gel), R_f (chloroform 92.3%; acetone 7.7%) 0.23 and by mixed melting point with an authentic sample. Concentration of mother liquor gave an additional crop of crystals, 0.008 Gm. (6.40%), m.p. 216°. The total yield was 0.063 Gm. (50.4%).

N-Phenoxycarbonyl-2-imidazolidone (XXI).—A 1.56-Gm. (9.96 mmoles) quantity of phenyl chloroformate was added to a stirred solution of 0.860 Gm. (9.99 mmoles) of 2-imidazolidone in 12 ml. of chloroform. The reaction mixture was heated under reflux until the evolution of hydrogen chloride ceased (45 hours). Concentration of the reaction mixture to half volume *in vacuo* gave white crystals which were collected by filtration. A 1.10-Gm. (53.5%) quantity of white crystalline material, m.p. 186–187°, was obtained. Concentration of the mother liquor gave a second crop of 0.400 Gm. (19.5%) of the desired product, m.p. 189°; total yield: 73%. One recrystallization of the crude product from chloroform gave the analytical sample, m.p. 189°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3300 (NH); 1795 and 1695 (C=O); 1595 and 1490 (phenyl); $\lambda_{max.}^{ethanol}$ 274 mμ ($\epsilon \times 10^{-3}$) 5.80.

Anal.—Calcd. for $C_{10}H_{10}N_2O_3$: C, 58.24; H, 4.88; N, 13.58. Found: C, 58.33; H, 5.14; N, 13.60.

N-Ethoxycarbonyl-2-imidazolidone (XXV).—To a stirred solution of 1.72 Gm. (19.9 mmoles) of 2-imidazolidone in 25 ml. of chloroform was added 2.17 Gm. (20 mmoles) of ethyl chloroformate. The reaction mixture was heated under reflux for 96 hours and evaporated to dryness *in vacuo*. The residue was crystallized with water which gave the white crystalline product, 1.10 Gm. (34.9%), m.p. 130°. Concentration of the mother liquor gave an additional 0.540 Gm. (17.1%) of white crystalline material, m.p. 130° total yield: 52.0%. Two recrystallizations of the crude product from water gave the analytical sample, m.p. 130°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3255 and 3020 (NH); 1800 and 1680 (C=O).

Anal.—Calcd. for $C_6H_{10}N_2O_3$: C, 45.56; H 6.32; N, 17.71. Found: C, 45.77; H, 6.37. N, 17.67.

N-Methoxycarbonyl-2-imidazolidone (XXVIII).—A 0.945-Gm. (10 mmoles) quantity of methyl chloroformate was added to a stirred solution of 0.860 Gm. (9.99 mmoles) of 2-imidazolidone in 10 ml. of chloroform. The reaction mixture was heated under reflux until the evolution of hydrogen chloride ceased (42 hours); on concentrating to half volume *in vacuo* a white crystalline material appeared which was collected by filtration; yield: 0.500 Gm. (34.8%), m.p. 180°. Concentration of mother liquor gave a second crop of 0.400 Gm. (27.8%) of the desired product, m.p. 178°. The total yield was 62.6%. One recrystallization of the crude product from water gave the analytical sample, m.p. 180°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3380 (NH), 1760 and 1670 (C=O).

Anal.—Calcd. for $C_5H_8N_2O_3$; C, 41.66; H, 5.56; N, 19.44. Found: C, 41.85, H, 5.40; N, 19.49.

Reaction of N-Phenoxycarbonyl-2-imidazolidone and Piperidine (2 mmoles).—A mixture of 0.206 Gm. (1.00 mmole) of N-phenoxycarbonyl-2-imidazolidone (XXI) in 4 ml. of chloroform and 0.085 Gm. (1 mmole) of piperidine in 3.7 ml. of chloroform was heated under reflux for 6.45 hours after which time 1 mmole of piperidine was added to the reaction mixture. Heating of the reaction mixture was continued for another 8 hours, after which time the peak at 1795 cm.$^{-1}$ in the infrared spectrum had disappeared. The reaction mixture was evaporated to dryness *in vacuo*, and the yellow solid which was obtained (0.170 Gm., m.p. 156°) was recrystallized from water; yield: 0.092 Gm. (46.5%) of N-piperidinocarbonyl-2-imidazolidone (XXII), m.p. 181°. Two recrystallizations of the product from water gave the analytical sample. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3350 (NH); 1730 and 1680 (C=O); 1655 (NH).

Anal.—Calcd. for $C_8H_{15}N_3O_2$: C, 54.90; H, 7.56; N, 21.65. Found: C, 54.96; H, 7.35; N, 21.29.

Reaction of N-Phenoxycarbonyl-2-imidazolidone and Piperidine (Excess).—To 1.23 Gm. (6.00 mmoles) of N-phenoxycarbonyl-2-imidazolidone (XXI) was added 45 ml. of piperidine; the mixture was heated under reflux for 42 hours. On keeping the reaction mixture overnight at 0°, white crystals appeared which were collected by filtration. A 0.440-Gm. (26.0%) quantity of N,N'-bis-(N-piperidinocarbonyl)ethylenediamine (XXIII), m.p. 221°, was obtained. Evaporation of the mother liquor to dryness *in vacuo* and recrystallization of the residue from a methanol and water mixture gave 0.320 Gm. (18.9%), m.p. 221° of the product (XXIII). Three recrystallizations from a methanol and water mixture gave the analytical sample, m.p. 224°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3350 (NH); 1620 (C=O).

Anal.—Calcd. for $C_{14}H_{26}N_4O_2$; C, 59.54; H, 9.28; N, 19.84. Found: C, 59.36; H, 9.45; N, 20.01.

Reaction of N-Phenoxycarbonyl-2-imidazolidone and Morpholine (Excess).—A mixture of 0.206 Gm. (1.00 mmole) of N-phenoxycarbonyl-2-imidazolidone (XXI) and 7.70 ml. of morpholine was heated under reflux for 30 minutes, after which time the peak at 1795 cm.$^{-1}$ had disappeared. The reaction mixture was evaporated to dryness; the white residue on recrystallization from a mixture of chloroform and hexane gave 0.117 Gm. (41.0%) of N,N' - bis - (N - morpholinocarbonyl)ethylenediamine (XXIV), m.p. 215°. Concentration of the mother liquor gave another crop of crystals, 0.042 Gm. (14.7%), m.p. 214°. Two recrystallizations of the crude product from a mixture of chloroform and hexane gave the analytical sample, m.p. 214°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3310 (NH); 1620 (C=O); 1550 (NH).

Anal.—Calcd. for $C_{12}H_{22}N_4O_4$: C, 50.05; H, 7.75; N, 19.60. Found: C, 50.12; H, 7.86; N, 19.77.

Reaction of N-Ethoxycarbonyl-2-imidazolidone and Piperidine (Excess).—A mixture of 0.158 Gm. (1.00 mmole) of N-ethoxycarbonyl-2-imidazolidone (XXV) and 7.70 ml. of piperidine was heated under reflux for 25 minutes, after which time the peak at 1800 cm.$^{-1}$ had disappeared. The reaction mixture was evaporated to dryness *in vacuo;* the residue after recrystallization from a hexane and chloroform mixture gave the white crystalline material 0.180 Gm. (74.1%), m.p. 153°, identified as

N - (N - piperidinocarbonyl) - N' - (ethoxycarbonyl) ethylenediamine (XXVI). One recrystallization from a mixture of methanol and water and another from a hexane and chloroform mixture gave the analytical sample, m.p. 149–150°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3440 and 3300 (NH); 1695 and 1620 (C=O); 1520 (NH).

Anal.—Calcd. for $C_{11}H_{21}N_3O_3$: C, 54.29; H, 8.70; N, 17.27. Found: C, 54.35; H, 8.70; N, 17.28.

Reaction of N-Ethoxycarbonyl-2-imidazolidone and Morpholine (Excess).—A mixture of 0.158 Gm. (1.00 mmole) of N-ethoxycarbonyl-2-imidazolidone (XXV) and 7.70 ml. of morpholine was heated under reflux for 25 minutes, after which time the peak at 1800 cm.$^{-1}$ in infrared spectrum had disappeared. The reaction mixture was evaporated to dryness *in vacuo;* the residue after recrystallization from a mixture of chloroform and hexane gave 0.110 Gm. (44.8%) of N-(N-morpholinocarbonyl)-N'-(ethoxycarbonyl) ethylenediamine (XXVII), m.p 156°. Concentration of the mother liquor gave another crop of crystals, 0.075 Gm. (30.3%), m.p. 156°. Recrystallization of the crude product from a mixture of methanol and water gave the analytical sample, m.p. 156°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3420 and 3300 (NH); 1705 and 1635 (C=O).

Anal.—Calcd. for $C_{10}H_{19}N_3O_4$: C, 48.96; H, 7.80; N, 17.13. Found: C, 49.25; H, 7.62; N, 17.15.

Reaction of N-Methoxycarbonyl-2-imidazolidone and Piperidine (Excess).—A mixture of 0.144 Gm. (1.00 mmole) N-methoxycarbonyl-2-imidazolidone (XXVIII) and 7.70 ml. of piperidine was heated under reflux for 20 minutes, after which time the peak at 1760 cm.$^{-1}$ had disappeared. The reaction mixture was evaporated to dryness *in vacuo,* and the residue on recrystallization from a mixture of chloroform and hexane gave 0.150 Gm. (65.7%) of N-(N-piperidinocarbonyl)-N'-(methoxycarbonyl)-ethylenediamine (XXIX), m.p. 134–136°. One recrystallization from a chloroform and hexane mixture and another from a mixture of water and methanol gave the analytical sample, m.p. 136°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3450, 3305, and 3100 (NH); 1710 and 1630 (C=O); 1560 and 1540 (NH).

Anal.—Calcd. for $C_{10}H_{19}N_3O_3$: C, 52.38; H, 8.35; N, 18.32. Found: C, 52.46; H, 7.93; N, 18.28.

Reaction of N-Methoxycarbonyl-2-imidazolidone and Morpholine (Excess).—Morpholine (7.70 ml.) was added to 0.144 Gm. (1.00 mmole) N-carbomethoxy-2-imidazolidone (XXVIII), and the mixture heated under reflux for 45 minutes, after which time the peak at 1760 cm.$^{-1}$ in infrared spectrum had disappeared. The reaction mixture was evaporated to dryness *in vacuo;* the residue on recrystallization from a chloroform and hexane mixture gave 0.150 Gm. (64.9%) of N-(N-morpholinocarbonyl) - N' - (methoxycarbonyl) - ethylenediamine (XXX), m.p. 149–152°. One recrystallization of the product gave the analytical sample, $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3505, 3390, and 3175 (NH); 1725 and 1650 (C=O); 1555 (NH).

Anal.—Calcd. for $C_9H_{17}N_3O_4$: C, 46.74; H, 7.41; N, 18.17. Found: C, 46.39; H, 7.56; N, 17.90.

Reaction of N-(N-Piperidinocarbonyl)-2-imidazolidone (XXII) and Piperidine (Excess).—A mixture of 0.098 Gm. (0.50 mmole) of N-(N-piperidinocarbonyl-2-imidazolidone and 5.70 ml. of piperidine was heated under reflux for 6.5 hours, after which time the peak at 1730 cm.$^{-1}$ in infrared spectrum had disappeared.

The reaction mixture on cooling to room temperature gave 0.069 Gm. (42.5%) of N,N'-bis-(N-piperidinocarbonyl)ethylenediamine (XXIII), m.p. 222°. Concentration of the mother liquor gave an additional 0.03 Gm. (21.2%), m.p. 222–223° of the product. The total yield was 0.09 Gm. (63.7%).

Reaction of N - (N - Morpholinocarbonyl) - N' - (ethoxycarbonyl) - ethylenediamine (XXVII) and Morpholine (Excess).—A 7.80-ml. quantity of morpholine was added to 0.07 Gm. (0.28 mmole) of N-(N-morpholinocarbonyl)-N'-(ethoxycarbonyl)-ethylenediamine (XXVII). The reaction mixture was heated under reflux for 46 hours, after which time the peak 1705 cm.$^{-1}$ in infrared spectrum had disappeared. Evaporation to dryness *in vacuo* of the reaction mixture and recrystallization from a mixture of chloroform and hexane gave 0.03 Gm. (31.2%) of N,N'-bis-(N-morpholinocarbonyl)ethylenediamine (XXIV), m.p. 214°.

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